REMARKS

Status of the Claims

Claims 1-18 and 21-22 are now pending. The Examiner's comments are addressed below in the order set forth in the Office Action dated November 24, 2003.

The Rejections of the Claims Under 35 U.S.C. § 103 Should Be Withdrawn

Claims 1-16, 21, and 22 stand rejected under 35 U.S.C. § 103 over Li *et al.* (1998) *BioTechniques* 25:358-361 in view of Patel *et al.* (1991) *Nucleic Acids Res.*, 19:3561-3567 and Michalatos-Beloin *et al.* (1996) *Nucleic Acids Res.* 24:4841-4843. This rejection is respectfully traversed.

Li *et al.* describes a method of haplotyping a segment of genomic DNA containing two or more polymorphisms. The method of Li *et al.* involves three general steps: Generating a linear heterozygous PCR product from the DNA segment; circularizing the segment by intramolecular ligation; and performing allele specific inverse PCR (ASIP).

The rejection is based upon two premises regarding the teachings of Li *et al.* that are not scientifically accurate: *Premise 1.* That Li *et al.* teaches a method of haplotype determination that involves isolating a target sequence containing polymorphisms followed by bringing the polymorphisms into closer proximity via circularization. *Premise 2.* That Li *et al.* suggest an unsolved problem,[1] namely that there are polymorphisms too distant in DNA to be haplotyped by standard PCR, and that the ordinary practitioner would be motivated to use the long-range PCR of Michalatos-Beloin *et al.* to solve this problem. Both of these premises are unfounded, as described in the following paragraphs.

Premise 1. The Office Action states that the method of Li *et al.* involves utilizing PCR to amplify a DNA fragment from genomic DNA, then "ligating the ends of the DNA fragment to each other so as to produce a circular DNA molecule..., *wherein the ligation brings the first and second polymorphisms into closer proximity on the circular DNA molecule.*" See the Office

[1] To the extent the Examiner has maintained this point, Applicants' representative notes that the identification of an unsolved problem is an indicia of *nonobviousness*.

Action mailed 11/24/2003, page 2 (emphasis added). A review of Li *et al.* reveals that their technique *does not* bring polymorphisms into closer proximity on the circular DNA molecule.

Drs. McDonald and Evans explain this in their declaration, as follows.

> Li *et al.* does not circularize in a way that brings polymorphisms closer together the way our claimed method does. Figure 1 of our application and Figure 1 of Li *et al.* clearly demonstrates this result. When in linear genomic DNA, the distal-most polymorphisms, M-G/T in intron 1 and M(G/T)-N(AG) in exon 2, are separated by 30-43 bp (page 358, second column, line 12). Once circularized by Li *et al.*, the polymorphisms are the *same* distance from each other in one direction of the circle and *farther apart* in the other direction (a 357 bp linear molecule was circularized, thus 357 minus 30-43 gives the polymorphisms a distance of between 314 to 327 bps from each other).
>
> Li *et al.* does not provide the advantages of our method. By using inverse PCR, Li *et al.* simply generate back the same, or nearly the same, linear molecule that was circularized by using inverse PCR (after inverse PCR off of a 357 bp circle, Li *et al.* generate a 351 and a 366 bp fragment). In my scientific opinion, had Li *et al.* intended haplotype analysis of circular molecules the way our method allows (i.e. aimed at analyzing polymorphisms that had been brought into closer proximity), then Li *et al.* would *not* have used inverse PCR because inverse PCR simply generates back the same, or nearly the same, linear molecule that was circularized in the first place.

See the declaration of Drs. McDonald and Evans, ¶ 8, filed concurrently herewith. Li *et al.* does *not* bring polymorphisms into closer proximity by its circularization step.

Premise 2. The Office Action states: "Li suggests the use of the method on haplotyping distances that are too long to be PCR amplified (see page 361, column 1)" and "[a]n ordinary practitioner would have been motivated to use long range PCR to prepare the template for the method of Li...." See the Office Action, pages 4 and 5. The teaching of Li *et al.* does not support this premise.

Li *et al.* does not suggest the use of their own method for haplotyping polymorphisms that are separated *in genomic DNA* by distances that are too long to be PCR amplified. Drs. McDonald and Evans explain this point, as follows.

> Li *et al.* concludes with the following statement: "Although these alleles can be typed by allele-specific nested PCR following GPA-specific PCR, ASIP

[allele specific inverse PCR], rather then nested PCR, can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR." Li *et al.*, page 361, column 1. When the statement of Li *et al.* is placed in context, it is clear that Li *et al.* refers to haplotyping polymorphisms that are too distantly separated for the design of a single forward PCR reaction. To place the comment in context, one must consider the following two points: (1) The only problematic distance discussed by Li *et al.* is a separation of 30-40 base pairs which causes a primer design problem for forward allele-specific PCR, necessitating the use of allele-specific nested PCR. Li *et al.*, page 358, column 2. (2) Li *et al.* solves the problem by use of a circularization method that does not bring the polymorphisms closer (see paragraph 8, above) followed by inverse PCR. Conversely, Li *et al.* circularizes for the specific purpose of making the polymorphisms further apart so that AISP can be used rather than nested PCR. This is because the polymorphisms are too far apart for a single allele-specific primer to be designed for amplification, and too close together (30-43base pairs) for a single nested PCR amplifications to produce a band that could be visualized on standard agarose/ethidium bromide gels (it is common knowledge that it is very difficult to visualize bands less than 100 base pairs on these gels). By making the polymorphisms further apart, a single ASIP reaction can be performed that produces products that are long enough (351 base pairs and 366 base pairs) to be visualized on agarose gels. This is demonstrated by the fact that Li *et al.* circularizes to make the polymorphisms further apart in one direction, followed by inverse PCR that amplifies products of 351 base pairs and 366 base pairs which are much longer than the 30-43 base pairs that separates C/T and G/T. If Li *et al.* had intended to bring polymorphisms closer together, they would have done so by circularizing to bring the polymorphisms closer together for a single PCR primer to be designed, and they would have then used a forward, not an inverse PCR, off of the circularized template. In contrast, Applicants' method is designed to haplotype polymorphisms that are distantly located in *genomic* DNA by bringing the polymorphisms into closer proximity via circularization.

See the declaration, ¶ 9.

In greater detail, Li *et al.* faced several problems when haplotyping the MN blood group system (the subject of their article). First, the polymorphisms Li *et al.* utilized to type the M^G, M^T, and N alleles of the GPA gene also occurred in the GPB and GPE genes, which are over 95% homologous with the GPA gene. Li *et al.* solved this by carrying out GPA-specific PCR. Li *et al.*, page 358, column 2. Second, the M^G, M^T and N alleles cannot be typed by the single allele-specific PCR amplification technique. Li *et al.*, page 358, column 1. Li *et al.* explains the problem as follows.

> Although primers specific to M^G and N alleles can be designed, the M^T-(and N-) specific nucleotide T in intron 1 is located *too far* (30-40 bp upstream) from the three M- (M^G and M^T) specific bases in exon 2 to design a single M^T-specific primer.

Li *et al.*, page 358, columns 1-2 (emphasis added). The inability to design a single M^T-specific primer can be overcome by allele-specific nested PCR after GPA-specific PCR. Li *et al.*, page 361, column 1. However, Li *et al.* chose to use circularization and one round of ASIP, which allows for a *single* procedure using allele-specific primers. Li *et al.*, sentence spanning pages 360-1. Li *et al.* correctly notes on page 361 that multiple allele-specific PCRs (nested necessarily refers to multiple) can be performed for haplotpe analysis after GPA-specific amplification, so the statement on page 361 refers back to the problem on page 358, in that "AISP [a single procedure]...can be applied to haplotyping polymorphisms separated by a distance that is too far to be amplified by PCR." It is my scientific opinion that this sentence refers to the fact that the polymorphisms are separated by a distance too large for a *single* primer to be designed that could anneal to a region that contains multiple polymorphisms (in intron1 and exon2 here) for a *single* allele-specific PCR procedure (and thus requiring either nested/multiple PCRs or Li *et al.*'s single AISP method). PCR here means a single PCR reaction, rather than if they had said nested PCR, which requires multiple PCR reactions. Thus, the problem of distance Li *et al.* refers to is polymorphisms being too far apart to design a primer for a single PCR procedure, and not a problem of polymorphisms being too far apart in genomic DNA for conventional haplotype analysis, as Li *et al.* notes that these polymorphisms can be haplotyped by RFLP and SSCP. Li *et al.* recognizes the following three problems of distance:

1. Too far for primer design for a single PCR reaction
2. Too close for visualizing short products of allele-specific PCRs on a gel
3. Having to perform multiple nested PCRs to overcome these 2 distance problems

> Li *et al.* circumvents the need for multiple nested PCRs by circularizing to make the polymorphisms farther apart, followed by a single ASIP procedure that does not require a primer to anneal to a region containing multiple polymorphisms and produces products that are long enough to clearly see on a gel.

See the declaration, ¶ 10.

In the preceding response, Applicants pointed out that Li *et al.*'s statement was more plausibly interpreted to intend a situation in which neither long range nor standard PCR could be utilized. Further, Applicants contended that the Office Action's reliance upon the less plausible interpretation was founded upon hindsight reasoning using Applicants teaching. If the more plausible interpretation is adopted, then the rationale for combining Li *et al.* with Michalatos-Beloin *et al.* evaporates.

The present Office Action now asserts that if Li *et al.* had meant long range PCR, they would have specified as much. The Office Action reasons that "[Li *et al.*] expressly teaches separation that is too far apart for standard PCR" and concludes that "[s]ince Standard PCR can easily amplify 1000 or more bases, Li is discussing polymorphisms that are at least that distant...." See the Office Action, page 10. This conclusion is neither taught nor suggested by Li *et al.* or the art. It is based solely on the Examiner's assumption. Applicants invite the Examiner provide an affidavit to support his contention, as is required by 37 CFR 1.104(d)(2).

Moreover, the Office Action's premise that Li *et al.* identify an unsolved problem is not supported by the reference. Drs. McDonald and Evans explain this point in the following statement.

> It is my scientific opinion that Li *et al.* performs circularization and ASIP to circumvent the need for allele-specific nested PCRs from genomic DNA, not to bring polymorphisms closer together. In other words, the last statement by Li *et al.* means that ASIP should be used in place of PCR, not combined with PCR.

See the declaration, ¶ 11. Li *et al.* teaches *away* from combining with any other method because it teaches that ASIP can be used in place of PCR (long PCR or regular PCR) when polymorphims are distantly separated.

To summarize, given the differences between Li *et al.* and the presently claimed method enumerated by Drs. McDonald and Evans, it is clear that Li *et al.* fails to teach or suggest Applicants' claimed method. Further, because Li *et al.* solve their distance problem by use of ASIP, there is no motivation within Li *et al.* for its combination with the other references asserted in the Office Action.

The Office Action cites statements found in the secondary reference to support the combination of Li *et al.* with either Patel *et al.* or Michalatos-Beloin *et al.* However, as explained in the following paragraphs, these statements would not motivate one of skill in the art to make the combination asserted in the Office Action.

For instance, the rejection states that "Patel teaches that inverse PCR methods such as those used by Li can be applied to haplotype sequences up to 10 kb apart...." However, Applicants' representative has reviewed Patel *et al.* carefully and can not determine where Patel *et al.* refer to a method like that of Li *et al.* Patel *et al.* merely make a general statement indicating that "others have successfully applied inverse PCR to genomic regions of over 10 kb," followed by a citation to Earp *et al.* (1990) *Nucleic Acids Res.* 18:3271-3279.[2] The application of inverse PCR described in Patel *et al.* involves the digestion of total genomic DNA with a first restriction enzyme, circularization of the DNA, relinearization of the circular molecules using a second restriction enzyme, ultimately followed by an inverse PCR technique upon the relinearized genomic DNA molecules. There is no reference in Patel *et al.* to the method of Li *et al.*, which involves generating a linear heterozygous PCR product from a genomic DNA segment, circularizing the segment by intramolecular ligation, and then performing ASIP on the circularized molecule. Thus, there is no motivation in Patel *et al.* to combine these two dissimilar methods.

With respect to Michalatos-Beloin *et al.*, the rejection states that the reference "teaches haplotyping where the molecules are prepared by long range PCR" and that an "ordinary

[2] Applicants' representative has reviewed the reference to Earp *et al.* (1990) *Nucleic Acids Res.* 18:3271-3279. It describes a technique like that of Patel *et al.* wherein isolation of the target sequence proceeds *after* circularization and relinearization of restriction digested total genomic DNA.

practitioner would have been motivated to use long range PCR to prepare the template for the method of Li...." Applicants disagree.

The technique of Michalatos-Beloin *et al.* is a one-step PCR technique where haplotyping is accomplished using the same PCR step by which hemizygous target DNA is isolated. Thus, the hemizygous target DNA isolated by Michalatos-Beloin *et al.* would not be suitable for the technique of Li *et al.* which relies upon the isolation of a *heterozygous* PCR product. The Office Action overlooks this fundamental difference, relying upon the statement that "The allele-specific long range PCR products were used as templates for amplification of the STR." As explained on page 4867 of Michalatos-Beloin *et al.*, the amplification of a short terminal repeat (STR) from the target DNA was a control PCR used to confirm that the one-step long-range PCR technique truly isolated hemizygous target DNA. The rejection does not identify any motivation for the modification of Michalatos-Beloin *et al.* to accommodate the additional, different steps of Li *et al.*

Indeed, the Michalatos-Beloin method teaches that its chief advantage is that haplotyping *is conducted* by a single-step PCR protocol and that one need only analyze the PCR products of the Michalatos-Beloin method to determine the haplotype of the source DNA, stating "The ability to isolate hemizygous DNA segments readily from heterozygous genomes via molecular haplotyping will provide the accuracy necessary in these diverse applications' (page 4867, column 2)." Michalatos-Beloin *et al.* teaches away from its combination with methods that require additional steps to analyze the haplotype of a sequence of interest. Accordingly, the art worker would be motivated to utilize Michalatos-Beloin *instead* of Li *et al.* (or Patel *et al.*, for that matter), not to combine the two.

The Office Action now asserts that Applicants are conducting a "piecemeal examination of the references" and that the inquiry is "based upon the analysis of the Graham v. John Deere factors." However, it is the burden of the Office to first establish a basis for the combination of cited references. When patentability turns on the question of obviousness, the search for and analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness. *See, e.g., McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1351-52, 60 USPQ2d

1001, 1008 (Fed. Cir. 2001) ("the central question is whether there is reason to combine [the] references"). *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988) ("'teachings of references can be combined only if there is some suggestion or incentive to do so.'"). The Office Action states that "[i]n the current case, there is a specific problem which is recognized by Li, and for which a solution is provided by Patel and Michaelos-Beloin." For the reasons of record, a basis for the asserted combination has not been established and the rejection should be withdrawn accordingly.

Even if the motivation to combine the references existed--which it does not--the combination asserted within the Office Action would not produce Applicants' claimed method. Drs. McDonald and Evans explain the basis for this conclusion in the following paragraphs.

> The method of Patel *et al.* (1991) *Nucleic Acids Res.*, 19:3561-3567 is conceptually and technically different from our method. These differences include the fact that our claimed method isolates the target sequence from genomic DNA before circularization (ligation) by amplifying a DNA fragment by PCR. In contrast, the Patel *et al.* method isolates the target sequence after circularization of total genomic DNA. Further, our claimed method involves isolation of target sequence from undigested and unligated genomic DNA by PCR techniques, including long range PCR. In contrast, Patel *et al.* isolates target sequence via short-range Allele-specific PCR from digested and ligated genomic DNA and production of a hemizygous target sequence. See the final slide of our Communication filed February 11, 2003 (hereinafter "Slide 4," provided for the reader's convenience as Appendix A of the response filed concurrently herewith).

See the declaration, ¶ 6.[3]

Because of these differences, the method of Patel *et al.* could not be combined with Li *et al.* (or Michalatos-Beloin *et al.*) to produce Applicants' method, as explained by Drs. McDonald and Evans in the following statement: "Patel *et al.* cannot be combined with these other references to produce our method because Patel *et al.* teaches circularization of genomic DNA before isolation of the target (unlike Michalatos-Beloin *et al.* and Li *et al.*, which both isolate the target first)." See the declaration, ¶ 15.

[3] The Examiner conceded to the conceptual differences between the technique of Patel et al. and Applicants' claimed invention on page 9 of the Office Action mailed July 7, 2003.

Similarly, the combination of Li *et al.* with Michalatos-Beloin *et al.* asserted in the Office Action does not produce Applicants' invention. They explain the basis for this conclusion in their declaration as follows.

> The method of Michalatos-Beloin *et al.* (1996) *Nucleic Acids Res.* 24:4841-4843 is conceptually and technically different from our method. These differences include the fact that our initial isolation of the target sequence produces heterozygous target sequence, whereas Michalatos-Beloin *et al.* produces hemizygous DNA. Thus, we are not limited to haplotype analysis via isolation of hemizygous DNA. Michalatos-Beloin *et al.* themselves emphasized that their method produces hemizygous DNA by stating at page 4867, column 2, that "the ability to isolate hemizygous DNA segments from heterozygous genomes via molecular haplotyping will provide the accuracy necessary in these diverse applications." See the third slide of our Communication filed February 11, 2003 (hereinafter "Slide 3," provided for the reader's convenience as Appendix A of the response filed concurrently herewith).

See the declaration, ¶ 7.

> It is my scientific opinion that the combination of the methods of Li *et al.* and Michalatos-Beloin *et al.* does not produce our claimed method. If one were to generate hemizygous templates with Michalatos-Beloin, then perform the circularization and ASIP of Li, it would not generate a shorter fragment with the polymorphisms closer together because (1) the circularization step does not bring the polymorphisms closer and (2) Li *et al.'s* PCR primers for the ASIP are in opposite orientation from ours (compare Li, Figure 1 with Figure 1 of our application). This in itself is conceptually *different* from our method, as we do not perform inverse PCR from circularized templates. It is impossible to generate the fragments for haplotype analysis that our method does by using inverse PCR off of circular molecules.

See the declaration, ¶ 12.

The Office Action now contends that the relevant inquiry is not whether the combination of references would work. However, the Federal Circuit has ruled that a combination of references inoperable for its intended purpose teaches away from combining the same. *Tec Air Inc. v. Denso Manufacturing Michigan Inc.*, 52 U.S.P.Q.2d 1294, 1298 (Fed. Cir. 1999). Further, the mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification. *See In re Gordon*, 221 U.S.P.Q. 1125 (Fed. Cir. 1984). For the reasons already of record, the art fails to

provide the motivation for its combination, let alone suggest the modifications asserted in the Office Action.

For all of the reasons stated above, it is clear that the teaching, motivation, or suggestion to select and combine the references has not been demonstrated. Applicants respectfully request that the rejection of claims 1-16, 21, and 22 be withdrawn.

Claim 17 is rejected under 35 U.S.C. § 103 over Li *et al.* in combination with Patel *et al.* and Michalatos-Beloin *et al.* in further view of Krynetski *et al.* (1995) *Proc. Natl. Acad. Sci.*, 92:949-953. Applicants respectfully traverse.

As described above, there is no motivation to combine Li *et al.* with Patel *et al.* and Michalatos-Beloin *et al.* Krynetski *et al.* merely teaches a point mutation of the TPMT gene and does not satisfy the deficiencies of the primary references. Consequently, the motivation to combine these four references has not been established and the rejection should be withdrawn.

Claim 18 is rejected under 35 U.S.C. § 103 over Li *et al.* in combination with Patel *et al.* and Michalatos-Beloin *et al.* in further view of Martin *et al.* (2000) *Am. J. Hum. Genet.*, 67:383-394. Applicants respectfully traverse.

There is no motivation to combine Li *et al.* with Patel *et al.* and Michalatos-Beloin *et al.* Martin *et al.* teaches SNPs in the region surrounding the APOE gene but does not satisfy the deficiencies of the primary references. Accordingly, the motivation to combine these four references has not been established and the rejection should be withdrawn.

CONCLUSION

In view of the aforementioned amendments and remarks, Applicants respectfully submit that the objection to the specification and the rejections of the claims under 35 U.S. C. § 103 are overcome. Accordingly, Applicants submit that this application is now in condition for allowance. Early notice to this effect is solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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